THE LIMITING HYDROGEN-ION CONCENTRATION OF VARIOUS TYPES OF PNEUMOCOCCI

H. M. JONES

From the Department of Pathology and Bacteriology, University of Illinois, College of Medicine, Chicago

The final reaction produced by an organism when grown in a medium containing a utilizable carbohydrate is known as the limiting hydrogen-ion concentration of that organism. In a previous paper [1] it was shown that this reaction varies for a given organism, depending on factors which favor or hinder abundant growth. As the growth of the pneumococcus is markedly influenced by slight changes in environment, it was necessary to establish rather definite conditions under which the final H-ion concentrations were developed.

Glucose broth, with the initial reaction of P_H 7.0, is commonly used in making final H-ion concentration determinations, but in dealing with such delicately growing organisms as the pneumococcus, this medium was found unsuited for the purpose, because of the irregularity with which growth was obtained with some of the strains.

Table 1 shows the wide variations in the final H-ion concentrations of a number of strains of each of the four types of pneumococcus, when such a medium was used.

TABLE 1

INOCULATIONS FROM RAPIDLY GROWING 24-HOUR BLOOD-AGAR CULTURES MADE INTO BROTH CONTAINING 1% PEPTONE, 0.3% BEEF EXTRACT, 0.7% NaCl, 1% GLUCOSE, AND HAVING AN INITIAL REACTION OF PH 7.0

Organisms	Viability	Final PH
1	+	6.6
2	+	5.8
3	+	6.4
4	+	5.8
1 R	+	5.7
2 R	—	7.0
3 R	+	5.6
4 R	+	5.7
1 Vir.	+	6.2
2 Vir.	—	7.0
3 Vir.	+	6.4
4 Vir.	+	6.6
1 Vac.	+	6.0
2 Vac.	+	6.5
3 Vac.	+	5.7
4 Vac.	+	5.6

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[1] Jones, H. M.: Jour. Infect. Dis., 1920, 26, p. 160.

"Viability" of the organism in table 1 does not imply growth. It was proved by simply plating on blood-agar at the end of the four-day incubation period. The H-ion concentration determinations were made by the hydrogen electrode described elsewhere.[2]

None of the strains developed a greater final concentration than P_H 5.6. Lack of growth macroscopically was no indication of the lack of change in reaction; and visible growth was, likewise, no indication that the P_H had reached its highest possible concentration. To show the effect on the limiting H-ion concentrations, and also in diminishing these wide variations in P_H shown in table 1, contrast the results shown in table 2, in which the same series of organisms were inoculated into broth differing from that used in the previous experiment only in having an initial reaction of P_H 7.6, instead of P_H 7.0.

TABLE 2

INOCULATIONS FROM 24-HOUR BLOOD-AGAR SLANTS MADE INTO 1% GLUCOSE BROTH HAVING AN INITIAL REACTION OF PH 7.6

Organisms	Viability	Final PH
1	+	5.2
2	+	5.3
3	+	5.6
4	+	5.4
1 R	+	5.0
2 R	—	7.6
3 R	+	5.1
4 R	+	5.3
1 Vir.	+	5.2
2 Vir.	+	5.2
3 Vir.	+	5.2
4 Vir.	+	5.1
1 Vac.	+	5.4
2 Vac.	+	5.0
3 Vac.	+	5.4
4 Vac.	+	5.2

A surprising difference in the abundance of growth was also noted. The range of the final H-ion concentration of this series was from P_H 5.0 to P_H 5.6, which is about the same range of final concentrations for hemolytic streptococci of virulent type. The hope of utilizing this method for the differentiation of the types of pneumococci from each other, or from the streptococci, was therefore abandoned.

A comparison of tables 1 and 2, however, very clearly shows how the final concentration of the H-ion is affected by the initial concentration. The initial alkaline reaction of P_H 7.6 was very obviously favorable to abundant growth, and abundant growth, in turn, was favorable to final concentrations which were more uniform throughout the series. Lord [3] noted that the final H-ion concentrations for his

[2] Ibid., 1919, 25, p. 262.

[3] Jour. Am. Med. Assn., 1919, 72, p. 1364.

series of pneumococcus strains were approximately within these same limits.

Cullen and Chesney,[4] however, found that "acidification during growth in beef infusion media proceeds until a P_H of about 7.0 is reached. At this point growth stops." The error of their findings is not difficult to explain when it is recalled that they did not add glucose to their medium. That growth does not stop at P_H 7.0 was easily shown by inoculating some of our strains into glucose broth with an initial reaction of P_H 6.8 and making plates of the cultures after one

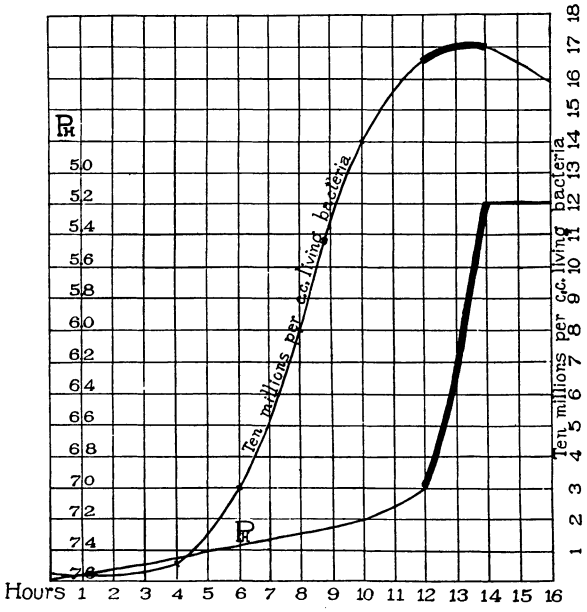

Fig. 1.—Curves of rate of change of P_H, coincident with rate of growth. Note that the greatest period of growth occurs while the reaction is still near neutrality, the interval indicated by light lines; and that the greatest period of sugar utilization occurs after the growth rate slows down — the interval indicated by heavy lines.

hour and again after 24 hours. Strain 3 R, for example, in 24 hours had increased from 24,000 to 52,000,000 viable bacteria per c c of culture. The P_H in the meantime had risen to P_H 5.9.

That their cultures developed an acid reaction in spite of the fact that glucose was not added to the medium, is explained by the fact that beef infusion medium contains considerable quantities of "muscle sugar." The final P_H reported by them would have been higher had

[4] Jour. Exper. Med., 1918, 28, p. 289.

they added more sugar, or lower if they had added a buffer, as disodiumphosphate. To obtain the final H-ion concentration of a given strain of bacteria, which will be reproducible in subsequent determinations, one should use a medium containing carbohydrate in excess of the amount which the given strain can remove from that medium.

A similar oversight is seen in the work of Dernby and Avery [5] who also used a beef infusion medium without addition of glucose. Their final concentrations would have been higher had glucose been added, as doubtless also would their bacterial counts. Their contention, that an initial reaction of about P_H 7.8 is the optimum for getting growth started is valid, but that growth does not continue at a P_H higher than 7.0 is not true, for then we should have here an obvious and simple basis for differentiation of this organism from Streptococcus hemolyticus. However, when abundant growth had occurred in a given culture in which an excess of glucose is present, the change in concentration of H-ion proceeds with such rapidity toward the higher ranges that the increase in growth is not proportionate. For example, in a culture of type 1, the P_H had risen from 7.6 to 7.0, the count had increased from 11,000 to 165,000,000 per c c in 12 hours, but in two hours more the P_H had suddenly risen to its maximum of P_H 5.2, while the count had had time to change only appreciably, as shown in figure 1.

TABLE 3

THE EFFECT OF AN INITIAL REACTION OF PH 7.0 IN HINDERING THE DEVELOPMENT OF PNEUMOCOCCI INTRODUCED, WITH 2 C C OF PURE BLOOD, INTO 100 C C OF GLUCOSE BROTH

Strain	Glucose Broth Initial PH 7.0 PH After 24 Hours	Growth per c c After 24 Hours	2% Blood in Glucose Broth Initial PH 7.0 PH After 24 Hours	Growth per c c After 24 Hours
1	6.6	15,000	4.8	800,000,000
2 Vac.	6.5	23,000	4.8	1,200,000,000
4 Vir.	6.6	2,500	5.0	2,000,000,000

This inability of the pneumococcus to grow in a medium of the usual reaction employed in ordinary bacteriologic mediums, namely P_H 7.0, is pronounced in some strains. For example, strains 1 of type 1, 2 Vac. of type 2, and 4 Vir. of type 4, produced no visible growth in such a medium, and, as seen in table 1, only very slight change in the P_H of the medium. The fact that blood cultures in pneumococcic septicemia often fail, seemed probably due to the fact that a broth of this unfavorable reaction may prevent the development of organisms, even though they may have been present in the blood under

[5] Ibid., p. 345.

examination. Accordingly, the effect of two parts of pure blood when introduced into 100 parts of glucose broth of this reaction, was tested, using those strains which grew poorly in glucose broth of P_H 7.0. Incidentally, the addition of the blood was shown to have no measurable effect in changing the reaction of the medium.

The results show that the introduction of 2% of blood is sufficient to cause profuse growth, even at the same reaction, P_H 7.0, at which growth in ordinary glucose broth fails. The experiment was repeated, using ascitic fluid in place of blood. In general, the effect was the same, both as to the abundance of growth, and the higher concentrations of H-ion developed by these strains. Long boiling renders these fluids useless in stimulating the profuse growths which resulted in the experiment described above.

In a previous paper [1] attention was called to the effect which these body fluids have in increasing the tolerance of Streptococcus hemolyticus of virulent type to the toxic ion of hydrogen. In other words, when these fluids are added to glucose broth, which is frequently done to insure growth, such a P_H is then developed that the virulent is made to imitate in P_H the avirulent types of streptococci. Table 3 show that the pneumococcus enjoys, with the streptococcus, this same increase of tolerance to the toxic ion of hydrogen, when these body fluids are added to the medium, which is of importance in connection with the fact that the virulent strains of streptococci are sometimes differentiated with great difficulty from the avirulent strains on the one hand, and the pneumococcus on the other.

No explanation of the action of the body fluids in stimulation of growth and increasing the organism's tolerance to hydrogen-ion, is attempted here, but since strong acidification, long boiling, etc., destroy this unknown "active principle," further search may reveal still other phenomena having "vitamins" as a basis of their explanation.

SUMMARY

The final hydrogen-ion concentration produced by pneumococci of various types when grown in glucose broth varies, with different strains, between P_H 5.0 and 5.6, being indistinguishable in this respect from various strains of Streptococcus hemolyticus of virulent type.

The regularity with which these final hydrogen-ion concentration values can be reproduced depends largely on the initial reaction, ordi-

nary glucose broth of P_H 7.0 being useless for this purpose. None of the strains failed to grow, however, when the initial reaction was set at 7.6.

This failure to grow in broth of P_H 7.0 does not account for the often observed failure to secure growth of the pneumococcus as when blood cultures are being made, since the addition of 2% of whole blood renders the medium of P_H 7.0 even superior to glucose broth of P_H 7.6 in stimulating growth.

A marked increase in tolerance toward the hydrogen-ion is also observed, as is also the case with Streptococcus hemolyticus.